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Article (Accepted Version)

Ghafarian, Farzaneh, Pashirzad, Mehran, Khazaei, Majid, Rezayi, Majid, Hassanian, Seyed Mahdi, Ferns, Gordon A and Avan, Amir (2019) The clinical impact of exosomes in cardiovascular disorders: from basic science to clinical application. *Journal of Cellular Physiology*, 234 (8). pp. 12226-12236. ISSN 0021-9541

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The clinical impact of exosomes in cardiovascular disorders: from basic science to clinical application

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Running title: exosome in CAD

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Grant: This study was supported by grant awarded (Amir Avan) by the Mashhad University of Medical Sciences.

Conflict of interest: The authors have no conflict of interest to disclose

Abstract

Cardiovascular disease (CVD) is the major cause of death globally; therefore, there is a need for the identification of valid biomarker that accurately predict the risk of developing cardiovascular disease, and novel therapeutic approaches for its treatment. Exosomes are very small extracellular vesicles containing protein, lipid, transcription factors, mRNAs, non-coding RNA and nucleic acid contents, that are important players in intercellular communication, and that act via long-range signals or cell-to-cell contact. The discovery of exosomes provides potential strategies for the diagnosis and treatment of cardiovascular disease. In the current review, we have explored the potential impact of exosomes on cardiovascular physiology, and their therapeutic potential in cardiovascular disorders with a emphasize on the existing preclinical studies.

Key words: exosomes, Cardiovascular diseases, nucleic acid contents, biomarker

Introduction

Cardiovascular disease (CVD) is a chronic disorder which is very common worldwide. Atherosclerosis, myocardial infarction (MI) and stroke are most prevalent categories of CVD (1). There are several classical risk factors contributing to the pathogenesis of CVD including age, sex, diabetes (2), cigarette smoking, systolic and diastolic blood pressure, and dyslipidemia (3).

Various types of cell therapy, including stem cells and cardiosphere-derived cells (CDC) have been used for promoting the repair and regeneration after CVD (4). A variety of cell types including embryonic stem cells, foetal cardiomyocytes and human umbilical cord-derived cells have been used as potential cell candidates for cardiac repair (5). Cardiopoietic stem cell therapy has been used in heart failure (HF) (C-CURE) as a potential new treatment for patients with HF of ischemic origin has been used, and myocardial remodeling, left ventricular ejection fraction (LVEF) levels were reduced by cell therapy (6). CDCs have recently been used for the treatment of heart failure and was also found to stimulate regeneration, angiogenesis and functional improvement (7). Mentowski et al engineered CDC-derived exosome, as an extracellular vesicle that was used to transport signaling molecules that play a critical role in intercellular communication, and expressed Lamp-2, an exosomal transmembrane protein. This was fused to a cardiomyocyte-specific peptide, to investigate the therapeutic potential of appropriate delivery to the heart. They have proposed that increasing the uptake of exosomes derived from CDCs by cardiomyocyte may be a suitable target for anti-apoptotic drug and gene therapy (8).

Furthermore, intercellular communication plays an important role in the survival and homeostatic cellular mechanisms. Intercellular communication can occur via direct contact or by signaling molecules secreted by cells. Gap junction proteins and cell surface interactions are important components of direct contact between cell-cell. These proteins can directly connect

two cells through cytoplasmic domains which leads to intracellular metabolite exchanges. Secreted signaling molecules including hormones, neurotransmitters and cytokines, bind to specific receptors which trigger specific cellular responses. Cell-cell communication can occur via another communication mechanism termed extracellular vesicle (EVs). These are released by, taken up by the cell membrane. The extracellular vesicle can deliver different signals to target cell via the binding of ligands to different cell surface receptors, delivering surface receptors and transferring signaling molecules such as lipids, proteins and RNAs (9). Three types of these vesicles are involved in various cellular processes, including: apoptotic vesicles, microvesicles and exosomes (10). Exosomes may fuse with endosomes to form complex structure named multivesicular bodies (MVBs). In turn, the fusion of these structures with cellular membrane results in the release of exosomes from these vesicles (11). The exosomes are nanosized secreted vesicles, which can execute intercellular communications, because they transport signaling molecules that include mRNA, siRNA and protein (12) (figure 1).

There has been increasing interest on the role of exosome composition in the causation of a variety of diseases such as cardiovascular disease (13). In addition, it has been shown that EVs, such as exosomes, play an important role in the pathogenesis of cardiovascular disease (14). Exosomes could also be an attractive biomarker for guiding therapeutic interventions in cardiovascular disease (14). Lai et al. examined how exosomes can stimulate the formation of mesenchymal stem cells (MSCs) in cardiac disease such as acute myocardial infarction. They also suggested that exosomes could be used as a biomarker for therapeutic agent in treatment of cardiac disease (15). This review summarizes the possible role of exosomes in the pathogenesis of cardiovascular disease including cardiomyopathy, MI, HF, and atherosclerosis.

Exosome formation

The fact that exosomes-containing particles may act as intercellular signaling molecules was first described in relation to maturation of sheep's reticulocytes (16). Exosome release from reticulocytes, can play a critical role in transferring various signaling molecules to cells or tissues. Furthermore, these vesicles have several receptors for other molecular components, for example a transferrin receptor. It has also been reported that transferrin receptor-containing exosomes can bind to the iron (Fe)-transferrin complex (17).

Many studies have showed that exosome formation is related to the destruction of some organelles, such as mitochondria and nucleus (18). It has been reported that exosome formation is increased following the maturation of reticulocytes via the release proteins that include Alix (19) during mitochondria destruction (18). To further support the relationship between exosome formation and organelles destruction, Fader et al. demonstrated that during differentiation of K562, a human erythroleukemia cell line to the erythrocytes, two factors enhance exosome formation that are derived from mitochondria destruction including Rab11, as a protein involved in fusion, and calcium (Ca^{2+}) (20). In contrast, it has been demonstrated that exosome formation during maturation of avian red cells is independent of mitochondria and nucleus destruction (19). It has also been found that differentiation of the erythroblast avian cell line to the red cells leads to secretion of transferrin receptor-containing exosome without loss of any organelles (21).

Biology of exosome synthesis and secretion

Apoptotic vesicles are between 1-4 μm in diameter, and contain signaling molecules that are destined for the cell membrane, cellular organelles and cytosol (10). Unlike Microvesicles which directly release from the plasma membrane, and are between 100nm to 1 μm , exosomes with budding to endosome vesicles form complex vesicles of MVBs (22). Unlike the two other types of extracellular vesicles, exosomal vesicles are between 40-100nm and are released by fusion

with MVBs with the plasma membrane which is associated with activation of exocytosis processes (23).

Different methods have been established for exosome formation and sorting of endosomal proteins into these vesicles. One of these methods is via the transport (ESCRT) pathway which is required for multivesicular bodies formation and sorting of endosomal components (24, 25). This transport system comprises several soluble multi-proteins including ESCRT-0, ESCRT-1, ESCRT-2, ESCRT-3 and accessory proteins. ESCRT-0 acts as a ubiquitin-dependent manner which is required for cargo clustering. ESCRT-1 and -2 can stimulate bud formation, whereas ESCRT-3 and accessory proteins such as vacuolar protein-sorting associated protein 4 (VPS4) ATPase, classifies vesicles and recruits deubiquitination machinery of the ESCRT system, respectively (24). It has been reported that there are 23 ESCRT components and its associated proteins may be silenced by using RNA interference which effects the production of antigen-containing exosomes (26). Some soluble multi-proteins and their associated proteins such as hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), TSG101 as an ESCRT-1 component and STAM1 as an ESCRT-0 components are depleted by RNA interference that leads to both a reduction of MHC-II-containing exosome production and effects the size and signaling molecules of exosome (26). To further support the role of soluble multi-proteins of ESCRT system in exosome formation, it has been reported that HRS, an ESCRT-0 component, is required both for exosome formation and its antigen-presenting capacity (27). Recently, in a study has examined the interaction between cellular components such as syndecan heparan sulfate proteoglycans and protein cytoplasmic adaptor of syntenin with protein ALIX, as a protein binding of ESCRT-3, and has been found to stimulate both exosome formation and the production of exosome-containing signaling molecules, such as CD63 in both primary dendritic cells (DCs) and C2C12, a muscle cell line (28, 29).

On the other hand, some studies have established that exosome formation is independent of the ESCRT transport system and dependent on natural cellular components (30, 31). In support of this, it has been reported that exosomes containing proteolipid proteins, tetraspanins and heat shock proteins are formed by silencing ALIX, HRS and TSG101 gene expression (32) using RNA interference, whereas secretion of some components such as proteolipid protein-positive exosome are controlled by ceramide production caused by natural sphingomyelinase-2 (33). In a study, sphingosine-1-phosphate (S1P), as a metabolite of sphingomyelin, are important in biogenesis of signaling molecules of exosomes such as CD63, CD81 and flotillin (31). In another study, it was shown that choline and phosphatidic acid are produced by phospholipase D activation which controls exosome secretion (34). Furthermore, lipid rafts, a normal cellular component, play an important role in exosome-sorting proteins (35). Therefore, it has been shown that markers of lipid rafts such as flotillin-1 (36), stomatin (37), GM1 and Lyn (38) have been identified in exosomes.

Exosomes are released by different types of cells in response to various biological and chemical stimuli. Rab GTPases (39), Wnt5a (40) and glycosphingolipids (41) are biological stimuli that lead to constitutive exosome secretion. Factors such as DNA damage (42), calcium release (43), heat shock proteins (44), thrombin (23), hypoxia condition (45), depolarization of plasma membrane and lipopolysaccharide (LPS) are chemical stimuli which result in inducible exosome secretion from the cell. In line with this, it has been shown that exosomes secretion are increased by different stimuli factors including oxidative stress (46), low pH (47) and hypoxia (48). It has also been reported that depolarization of plasma membrane caused by K^+ result in increased exosome release from the neural cells (49). Different factors that include: low levels of hydrogen peroxide (H_2O_2) can induce cardiomyocyte cells to releasing exosome from these cells (50). It has been established that changes in the cell environmental, such as ischemia can trigger the release of exosomes from the heart (50).

Molecules such as DNA, RNA and proteins are components of exosomes which can transfer signals to the target cells (51). It has been shown that exosomal proteins are common to the protein components of other cellular organelles including plasma membrane, cytosol, Golgi and nucleus (52). On the other hand, it has also been reported that lipid components such as cholesterol, sphingolipids, glycerophospholipids, phosphatidylcholine and phosphatidylethanolamine are highly enriched in exosome membrane compared with plasma membrane (53). Furthermore, it has also been demonstrated that exosome membrane is enriched of tetraspanins such as CD9, CD63, CD81 and CD82, GPI-anchored proteins and tumor necrosis factor receptor 1 (TNFR1) which are known as protein components of membrane exosome (54).

Consistent with these results, we suggested that exosome-containing signaling molecules and components changes can be an appropriate factor for examining etiology of diseases such as CVD.

Role of exosomes in cardiomyopathy

Cardiomyopathy or right ventricular dysplasia is a disorder of heart muscle (55) with an autosomal dominant mode of inheritance (56).

Aminzadeh et al. have shown that human CDCs-secreted exosomes improve the features of Duchenne's cardiomyopathy in mdx mice. They also demonstrated that exosomes released from CDCs improve cardiac function and overall survival, as well as enhancing maximal exercise capacity by transferring the miR-148a signaling molecule in this model (57). Suzuki investigated the protein biomarkers such as fibrinogen gamma chain (FGG) in plasma exosomes by mass spectrometry in model of dilated cardiomyopathy (DCM) in 4C30 mouse (58). Moreover, Jiang et al. showed that exosomes derived from the serum of children with DCM

stimulated gene expression-associated pathological changes in human-induced pluripotent stem cell-derived cardiomyocyte (iPSC-CMs) and neonatal rat ventricular myocytes (NRVMs) (59).

Gallet et al. possible use of percutaneous trans-endocardial delivery of CDCs using the NOGA MYOSTAR injection catheter in a post-MI cardiomyopathy model in pigs. They demonstrated that exosome secretion-related to the CDCs, inhibit left ventricular (LV) adverse remodeling and reduce scar mass and scar size in the pigs injected with CDCs (60).

Exosomes can improve the changes of cardiovascular system including reducing of Ca^{2+} response, mitochondrial dysfunction and reduce the response of the β -adrenergic receptor, via exosomal components, in septic cardiomyopathy. To further support the role of exosomes in the pathogenesis of septic cardiomyopathy, Monteiro et al. demonstrated that exosomes containing nicotinic adenine dinucleotide phosphate hydrogen (NADPH) induce cell death and cardiac dysfunction by internalization of exosomes in endothelial cells in septic cardiomyopathy (61).

Recent studies have shown that exosomes containing low heat shock protein 20 (Hsp 20) are released from cardiomyocytes derived from diabetics. Wang et al. examined the potential protective effect of exosomes containing high levels of Hsp20 against streptozotocin (STZ)-induced diabetes and adverse cardiac remodeling in a mouse model of cardiac-specific overexpression of Hsp20. They demonstrated that over-expression of exosomes derived Hsp20 attenuates cardiac dysfunction, hypertrophy, fibrosis, apoptosis, and microvascular rarefaction in STZ-treated mice, and increases exosomes secretion because of a direct interaction between Hsp20 and Tsg101, an initiator of exosome biogenesis in cells (62). Wang et al. also showed that exosomes containing high levels of Hsp20, enhance cardiac blood vessel density and reduce fibrosis of myocardial in STZ diabetic mice (63). Furthermore, exosomes containing Hsp20 stimulate cardiac angiogenesis in myocytes. Furthermore, Gu et al. have reported that exosomes containing high concentrations of Hsp20 improve cardiac dysfunction and

angiogenesis by increasing the interaction between Hsp20 and Tsg101 induced by overexpression of Hsp20 in model of STZ-induced diabetic TG and WT mice (64).

The role of exosomes in the atherosclerosis

Atherosclerosis is an inflammatory disease that may involve both innate and adaptive immune responses. There are several factors regulating the pathogenesis of atherosclerosis including immune cells such as monocytes, macrophages, dendritic cells, and lymphocytes, chemokines, interleukins, oxidized low-density lipoprotein (LDL) and HPSs (65).

Perrotta and Aquila, studied the presence of exosomes in human atherosclerotic plaque using transmission electron microscopy. They demonstrated that exosomes are formed by both endothelial cells and lesional smooth muscle cells (66).

Moreover, exosomes enhance inflammatory responses and vessel infiltration by delivering the contents of apoptotic and inflammatory between blood cells and vascular cells in atherosclerosis process (67). To further support the role of exosome in atherogenesis, Gao et al. investigated the effect of exosomes on endothelial inflammation and atherosclerosis by using a transwell model system and exosome release inhibitor GW4869. They stimulated human umbilical vein endothelial cell (HUVEC) with dendritic cell-derived exosomes and showed that exosomes secreted by dendritic cells and tumor necrosis factor (TNF)- α on cellular membrane of exosomes enhance HUVEC inflammation and atherosclerotic lesion by activation of the nuclear factor- κ B (NF- κ B) signaling pathway (68).

Exosomes containing miRNAs play a key role in the pathogenesis of atherosclerosis (69). Zheng et al. showed that exosomes containing miR-155 derived from vascular smooth muscle cells (VSMCs) stimulate endothelial permeability and progression of atherosclerotic plaque by disrupting the tight junctions between endothelial cells, and endothelial barrier integrity, which is

mediated by over expression of miR-155 induced by kruppel-like factor 5 (KLF5), a regulator of the intact endothelial barrier, in VSMCs (70). It has also been reported that exosomes containing long noncoding RNA HIF1 alpha-antisense RNA1 (lncRNA HIF1A-AS1) is important in atherosclerosis. Wang et al. examined the expression and concentration of exosome-derived lncRNA HIF1A-AS1 in atherosclerosis using quantitative real-time polymerase chain reaction (qRT-PCR) using plasma samples of patients with atherosclerosis and healthy adults. They demonstrated that the expression and concentration of exosomal lncRNA HIF1A-AS1 was higher in plasma samples from patients with atherosclerosis compared to healthy controls (71).

The role of exosomes in the heart failure (HF)

Heart failure (HF) is an inability of the heart to preserve normal cardiac output without increased venous filling pressure. The heart failure is characterized by the pulmonary edema and dyspnea and fatigue symptoms (72).

It has been shown that myocardial microvascular exosomes play an important role in cardiac homeostasis. Juni et al. demonstrated that cell-cell interaction between endothelial cells and cardiomyocytes is mediated by exosomes-derived signaling contents including vascular endothelial growth factor, nitric oxide, and non-coding RNAs. They also reported that exosomes transporting non-coding RNAs from endothelial to cardiomyocytes, and vice versa, regulate the angiogenic processes in the heart (73). Yang et al. investigated the relationship between exosomes-derived circulating miRNA and heart failure-associated myxomatous mitral valve disease (MMVD-CHF) by qRT-PCR using exosomes containing miRNA-extracted from plasma samples of dogs with MMVD-CHF. They showed that exosomes-derived miRNAs including miR-181c, miR-495, and miR-9 are highly expressed in plasma samples of dogs with MMVD-CHF (74). To further support the over expression of exosome-derived miRNAs in heart failure

process, Yamaguchi et al. reported that repeated remote ischemic conditioning (RIC) reduce MI-induced LV fibrosis by increasing expression of exosomes-derived miR-29a in RIC-treated rats (75).

Furthermore, it has been reported that injection of CDCs to heart failure-preserved ejection fraction (HFpEF) rats improves recovery of the diastolic function via reduction of inflammation and fibrosis. Cho et al. investigated the effect of CDC-derived exosomes on systolic and diastolic function assessed using transthoracic echocardiogram (ECHO) in model of HFpEF in Dahl salt-sensitive (DS) rats. They demonstrated that injected CDCs-derived exosomes to HFpEF rats reduce the E/E_v ratio which indicates an improvement in diastolic function (76).

The role of exosomes in the myocardial ischemia (MI)

Myocardial ischemia (MI) is the most common cause of death globally. An MI is detected by a changing pattern of serum troponin and electrocardiographic (ECG) findings (77, 78).

It has been shown that exosomes, can be protective during the ischemia and reperfusion injury that may be associated with a myocardial ischemia. Vicencio et al. investigated the cardioprotective effect of exosome-derived components on ischemia reperfusion by protein marker expression, nanoparticle tracking analysis, western blotting, and transmission electron microscopy using the isolated exosomes from rats and human blood samples. They demonstrated that cardiomyocyte-derived exosomes containing HSP70 protect against ischemia reperfusion injury via the binding of HSP70 to toll-like receptor (TLR) 4 which result in activation of HSP27 in cardiomyocytes (79). To further support the cardioprotective effect of mesenchymal stem cells (MSC)-derived exosomes against ischemia reperfusion injury, Lai et al. showed that exosomes secreted by MSCs attenuate infarct size by a paracrine effect in a myocardial ischemia reperfusion model in mouse (80). It has also been demonstrated that a

protective effect of exosomes derived from MSCs is mediated by cellular components including increasing the adenosine three phosphate (ATP) and nicotine adenine dehydrogenase (NADH) levels as well as enhancing the phosphorylation of both Akt and glycogen synthase kinase (GSK) 3 β , and reducing the phosphorylation of c-JNK after myocardial ischemia reperfusion injury in the C57Bl6/J mouse model (81). To further support the role of MSC-derived exosomes protective effect against myocardial ischemia reperfusion injury, Zhao et al. examined the protective effect of exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs) on cardiac function and apoptosis by using echocardiography and TUNEL staining, respectively, following an induced acute myocardial ischemia (AMI) in rats. They reported that exosomes derived from hucMSCs attenuate both cardiac dysfunction and fibrosis, as well as protect apoptosis of myocardial cells (82).

Zhang et al. showed that hypoxia increased the number of exosomes derived from H9C2 cells, without any changes in the size of exosomes. They reported that some exosomal miRs such as miR-152-3P and miR-21-5P were upregulated in hypoxic stress and exhibited anti-apoptotic effects. They proposed that some hypoxia stimulated exosomal miRs, such as miR-152-3p and let-7i-5p from H9C2 cells played an anti-apoptotic role through the mitochondrial and cell death pathways. Taken together, miRs of H9C2 cell-exosomes had cardioprotective effects under hypoxic-condition by inhibiting the apoptotic pathway (3).

Mesenchymal stem cell-derived exosome taken up by endothelial cells were shown to promote angiogenesis (18). The cardioprotective effects of MSC-derived exosomes may be associated with the stimulation of autophagy in H9C2 cells following stimulation of H₂O₂ hypoxia. The number of exosomes with 50-150 nm in diameter were increased after 24h of H₂O₂ treatment. MSC-exosomes appeared to protect H9C2 cells treated with H₂O₂. Internalization of the MSC-exosomes increased cell viability and autophagy, which led to attenuated apoptosis, and reduced ROS production (19). Gonzalez-King and colleagues have shown that the

concentration of exosomes was higher in mesenchymal stem cells cultured medium when treated with Hypoxia Inducible Factor-1 α (HIF-1 α), and the exosomal proteins were associated with tissue development and morphogenesis. However, exosomal proteins which secreted by HIF-treated MSCs were associated with stabilization and metabolic HIF-1 α process (20).

The characteristics of mesenchymal stem cells and exosomes derived MSC (MSC-exo) have been investigated. MSC expressing CD90 are spindle shape and originated from the bone marrow, the MSC-Exo cells were cup-shaped and express CD63. Both of MSC and MSC-exo could decreased inflammation, inhibited fibrosis and improved cardiac function in the rat myocardial infarction model. However, these effects were superior in MSC-Exos rather than MSC (21). Furthermore, the morphology of adipose-tissue derived MSC (ADMSC) and exosome derived from them has been characterized. ADMSC was spindle shaped, like MSC, with markers including CD29, CD44 and CD105. However, the markers of the exosome secreted from ADMSC included: CD9, CD63, CD 81 and HSP-70 (16).

Endometrium-derived mesenchymal stem cells (EnMSCs) could enhanced cardiac function after an MI via high exosomal expression in a rat model. Investigation of miR profiles in MSC derived from endothelium showed that miRs profile were different from adipose and bone marrow-derived MSCs. Among various EnMSC miRs including miR-1275, miR-21-5p, miR-23-3p, miR-3940-5p, miR-4708-3p, miR-548ap-5p, and miR-642b-5, and their expression was confirmed in EnMSC, miR-21 had a higher expression. miR-21 had protective effect against ischemic myocardium from apoptosis and stimulated angiogenesis. miR-23 in EnMSC-derived exosomes could promote angiogenesis in cardiac endothelial cells by activating proangiogenic signaling (7).

In addition, it has been shown that a cardioprotective effect of exosomes derived from cardiac progenitor cell (CPC) elicit oxidative stress through suppression of activated caspase 3/7 in

cardiomyoblasts cell lines, H9C2, as well as suppress apoptotic cardiomyocyte cells in model of myocardial ischemia/reperfusion in mouse *in vitro* and *in vivo*, respectively (83).

Sluijter and Rooij. examined correlation of the changes in exosomes containing miRNAs types derived from CPCs and biological effect of exosome during ischemia injury using analysis of miRNA expression profiles in exosomes in mice. They reported that exosomes derived from CPCs in hypoxic condition higher levels of several miRNA compared to cardiac progenitor cell-derived exosomes grown under normoxic conditions and identified 4 clusters of miRNA correlate to a biological effect related to the therapeutic benefit (84). Cervio et al. showed that exosomes containing miR-146, miR-210, and miR-132 derived from CPC elicit cardioprotective and proangiogenic effects on apoptosis and angiogenesis of cardiomyocytes via inhibition of apoptotic and induction of angiogenic these cells (85) (table1). Moreover, Khan et al. determined the effect of exosomes containing miRNAs derived from embryonic stem cells (ESCs) on MI using miRNA array in mouse. They reported that exosomes containing miR-294 increase both neovascularization and cardiomyocyte survival as well as reduce fibrosis post infraction (86).

The role of exosomes-derived components in cardiovascular function

It has been shown that endothelial-derived exosomes containing HSPA12B, a HSP70 family member, improves cardiovascular dysfunction induced by polymicrobial sepsis by increasing the expression of tight junction proteins affecting vascular permeability, inhibition of adhesion molecule expression and decreasing the infiltration of immune cells into the myocardium which is mediated by miR-126 derived from exosome (87).

Kang and coworkers showed that extracellular vesicles secreted from adipose-tissue derived stem cells (ASCs) promoted migration and tube formation *in vitro*. This proangiogenic effect is

mediated by miR-31 that targeted FIH1 in vascular endothelial cells. Most of the RNA in EVs were <200 nucleotides long. However, most RNAs in ASC are large. On the other hand, bioanalyzer RNA analysis demonstrated that cellular contamination is minimal which is associated with lack of 18s and 28s ribosomal RNA extracellular vesicles. In line with this, it has been reported that miRNA is enriched whereas almost all of small RNA (sRNA) is in a narrow range of 10-40 nucleotides (88). Therefore, several studies indicate that miR-31 is only proangiogenic miRNA (89, 90). Li et al. showed that the exosomes from endothelial progenitor cell (EPCs) exhibited angiogenic properties. These exosomes could be taken up by endothelial cells and activated angiogenic programs which exhibited its role in vascular regeneration. The pro-angiogenic genes including endothelial nitric oxide synthase (eNOS), interleukin-8 (IL-8), angiopoietin-1 (ANG-1) and E-selectin were increased more than 4-fold after treated cultured human microvascular endothelial cell lines (HMEC), with EPC-derived exosomes. Besides, VEGFA, VEGFR-2, hypoxia inducible factor 1 alpha (HIF-1 α), chemokine (C-X-C motif) ligand-16 (CXCL16) and platelet-derived growth factor alpha polypeptide (PDGFA) were upregulated by more than 1.5 fold (91).

Chang et al. identified that miR-146a-5p and miR-146b-5p could take up by endothelial colony-forming cells both in a free-form and within exosomes. Both inhibited angiogenic ability such as endothelial cell migration and tube formation (92). Other investigations also showed that injection of miR-155-rich exosomes into mice fed a high fat diet led to significant increase in miR-155 expression in the aortae and cause to plaque formation and severe impairment of endothelial integrity. On the other hand, adding of miR-155-rich exosomes to cultured endothelial cells, reduced the proliferation and migration of miR-155-rich exosome-treated endothelial cells, and also the ability of endothelial tube formation was lost. Therefore, miR-155 has anti-angiogenic effects on ECs. Furthermore, miR-155-rich exosomes led to a reduced expression of tight junction (TJ) proteins particularly zonula occludens-1 (ZO-1), so

mesenchymal stem cell derived miR-155 cause to damage the endothelial barrier function (4). In vitro investigation showed that neutral sphingomyelinase 2 (nSMase2) contributed to exosome secretion by human cardiosphere-derived cells (hCDCs) by triggering the budding of exosomes into multivesicular bodies. hCDCs have markers including CD90 and CD105 (mesenchymal markers), CD63 and HSP90 (exosomal markers), GATA4 and Nkx2.5 (markers of early cardiac development) and could be internalized by endothelial cells, cardiac fibroblasts and myocytes. hCDCs exosomes could increase endothelial migration, tub formation and branch point complexity, while reduced the human cardiac fibroblast proliferation (93). Secreted exosomes by cardiomyocytes in ischemic condition could increase mouse cardiac endothelial cell proliferation, endothelial cells migration, sprouting and capillary-like structures. Investigations showed that miR-222 of ischemic exosome is responsible for pro-angiogenic effects in exosome and led to tube formation and sprouting (94) (table2).

Beltrami and coworkers isolated exosomes from pericardial fluid (PF). They suggested that the cells of the heart and heart vessels could produce miRs which are transported to the PF via exosomes. PF-derived exosomes were able to induce the formation of capillary-like cellular network on Matrigel. Let-7b-5p is one the highly expressed miRs in the PF-exosomes. It induced angiogenesis and improved capillary-like tube formation on Matrigel (95).

Conclusion

Extracellular vesicles comprise apoptotic bodies, microvesicles and exosomes, and they perform as key regulators in cell-to-cell communication in normal as well as diseased states. The extracellular vesicles contain natural cargo molecules, such as miRNA, mRNA and proteins, and transfer these functional cargos to neighboring cells or more distant cells through circulation. These functionally active molecules then affect distinct signaling cascades. The message conveyed to the recipient cells is dependent upon the composition of the extracellular vesicle, which is determined by the parent cell and the biogenesis of the extracellular vesicle.

Because of their properties such as high stability and long half-life in the circulation, biocompatibility, low immunogenicity and toxicity, extracellular vesicles have attracted attention as putative delivery systems for therapeutics. It is interesting that exosomes from different cells may exhibit protective or destructive roles in cardiovascular diseases. The advanced technics to modify or load therapeutics into exosomes can be developed and standardized in a future study. It is undeniable that the unique opportunities and new challenges for characterization of exosomes as clinical biomarkers, diagnosis and prognosis factors in cardiovascular disease are an exciting prospect.

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Table 1 Exosomes and different kinds of cardiovascular diseases

cardiovascular diseases	Cell-derived exosomes	exosomes-derived contents	Mechanisms	Authors
Cardiomyopathy	CDCs	miR-148a	<ul style="list-style-type: none"> Improving the heart failure and survival as well as enhancing the maximal exercise capacity Suppression of LV adverse remodeling and reduction of scar size and scar mass 	<ul style="list-style-type: none"> Aminzadeh et al. (2015) Gallet et al. (2015)
	Endothelial cells	NADPH	Induction of cell death and cardiac dysfunction by internalization of exosomes	Monteiro et al. (2017)
	Overexpression of HSP20		<ul style="list-style-type: none"> Attenuation of cardiac dysfunction, hypertrophy, fibrosis, microvascular rarefaction via direct interaction between HSP20 and Tgs101 Enhancing the cardiac blood vessel density and reduction of myocardial fibrosis Improving the cardiac dysfunction and angiogenesis 	<ul style="list-style-type: none"> Wang et al. (2016) Wang et al. (2014) Gu et al. (2015)
Atherosclerosis	Dendritic cells		Enhancing the HUVEC inflammation and atherosclerotic lesion through NF-κB signaling pathway	Gao et al. (2016)
	VSMCs	miR-155	Stimulation of endothelial permeability and progression of atherosclerotic plaque by destroying the tight junction and endothelial barrier integrity	Zheng et al. (2017)
Heart failure	Endothelial cells	Non-coding RNAs	Regulation of angiogenic processes in heart	Juni et al. (2017)
	Plasma samples	miR-181c, miR-495, miR-9	Correlation between overexpression of these miRNAs and MMVD-CHF	Yang et al. (2017)
	Overexpression of miR-29a		Reduction of left ventricular fibrosis induced by MI	Yamaguchi et al. (2015)
Myocardial ischemia (MI)	Cardiomyocytes	HSP70	Protection of ischemia reperfusion injury via binding of HSP70 to TLR-4	Vicencio et al. (2015)
	MSCs		<ul style="list-style-type: none"> Attenuation of infarct size via paracrine effect of exosomes derived from MSCs Increasing the ATP and NADH levels, 	<ul style="list-style-type: none"> Lai et al. (2010)

		enhancing the phosphorylation both of Akt and GSK-3 β , reducing the phosphorylation of c-JNK	• Arslan et al. (2013)
	hucMSCs	Attenuation of function and fibrosis of cardiac and protection of apoptotic myocardia cells	Zhao et al. (2015)

Table 2 miRs of exosomes.

microRNAs	place	role	ref
miR-1	50% placed in Urine exosomes. 50% placed in urine supernatant.	Biomarker in AMI (both increased)	Cheng et al.
miR-24, 133a, 133b,	Increased after CABG surgery.	<ul style="list-style-type: none"> • Biomarker in surgery • Its elevation is associated with hs-cTn-I. 	Emanueli at al.
miR-210, 132	CPC-EV	Inhibition of apoptosis in HL-1 cells	Barile et al.
miR-451	CPC-exosome	<ul style="list-style-type: none"> • A GATA responsive miR • Inhibition of H₂O₂-induced apoptosis by reducing the level of caspase 3/7 	Chen et al.
miR-19a, miR-451	MSC with overexpression of GATA-4	<ul style="list-style-type: none"> • reduce infarct size • anti-apoptotic miR 	Yu et al.
miR-152-3p, miR-21-5p	H9C2 cell exosome	Anti-apoptotic	Zhang et al.
miR-23	EnMSC-derived exosomes	Promote angiogenesis	Ibrahim et al.
miR-146a-5p, miR-146b-5p	endothelial colony-forming cells	Inhibition of angiogenesis	Chang et al.
miR-155	MSC- exosome	<ul style="list-style-type: none"> • Inhibition of angiogenesis • Suppression of cardiac fibroblast proliferation • decrease the expression of tight TJ • suppressed the cardiac fibroblast proliferation • it promoted inflammation in cardiac fibroblast 	Zheng et al. Wang et al.
Let-7b-5p	PF-exosome	<ul style="list-style-type: none"> • Angiogenesis effect • Improve capillary-like tube formation 	Beltrami et al.
miR-31	ASCs-EV	Pro-angiogenic effect	Kang et al.
miR-222	Secreted exosomes by cardiomyocytes in ischemic condition	pro-angiogenic effect	Ribeiro-Rodrigues et al.
miR-29a	MI-induced rats with RIC exosome	anti-fibrosis effect	Yamaguchi et al.

Figure legend 1: schematic exosome synthase of process and different it's effects on cardiovascular disease

